SARS-CoV-2 Ag Self-test Kit (Colloidal Gold)

Instructions for Use (IFU)

For Lay Use
For in vitro Diagnostic Use Only
For Use with Saliva Specimens

Befristet zugelassen zur Eigenanwendung nach §11 MPG in Deutschland (BfArM GZ: 5640-S.032.21) ohne abgeschlossenes Konformitätsbewertungsverfahren.

This instruction for use (IFU) must be read carefully prior to use. Instructions for use must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions for use.

INTENDED USE
This kit is used for in vitro qualitative detection of Nucleocapsid(N) Protein antigen from SARS-CoV-2a human saliva samples. This kit is authorized for lay use with self-collected observed direct saliva samples from adults. Results are for the identification of SARS-CoV-2 Nucleocapsid Protein antigen. Antigen is generally detectable in saliva during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results should be treated as presumptive and confirmation with a molecular assay. Negative results cannot exclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results should be considered in the context of a patient’s recent exposure, history and the presence of clinical signs and symptoms consistent with COVID-19.

Individuals who test negative and continue to experience COVID-like symptoms should seek follow up care from their healthcare provider.

SUMMARY AND EXPLANATION OF THE TEST
Coronaviruses are a large family of viruses which may cause illness in animals or humans. SARS-CoV-2 is an enveloped, single-stranded RNA virus of the β genus. The virus can cause mild to severe respiratory illness and has spread globally.

The SARS-CoV-2 Ag Test Kit is a lateral flow immunosorbent for the qualitative detection of SARS-CoV-2 directly from saliva, without viral transport media. The Kit contains all components required to carry out an assay for SARS-CoV-2.

PRINCIPLE OF THE PROCEDURE
The SARS-CoV-2 Ag Test Kit is a lateral flow immunosorbent for the qualitative determination of Nucleocapsid Protein of SARS-CoV-2 virus in human saliva samples. SARS-CoV-2 nucleocapsid is immobilized in the test region on nitrocellulose membrane. If the specimen contains SARS-CoV-2 antigen, during the assay specimen is allowed to react with the colored conjugate (SARS-CoV-2 antibody- colloidal gold conjugate); the mixture then migrates chromatographically on the membrane by the capillary action. An SARS-CoV-2 positive specimen produces a distinct color band in the test region, formed by the specific antibody antigen colored conjugate complex “(Anti-SARS-CoV-2-Ab)+(SARS-CoV-2-Ag)- (SARS-CoV-2-Ag)”. Absence of this colored band in the test region suggests a negative result. A colored band always appears in the control region serving as procedural control regardless of the specimen contains SARS-CoV-2 or not.

REAGENTS AND MATERIALS

Materials provided

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Specification</th>
<th>components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test card with desiccant in a sealed foil pouch</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tube with sample extraction solution</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Disposable sterile swab</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Instruction for use</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Quick guide</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Materials required but not provided

- Clock, timer or stopwatch

PRECAUTIONS
1. For in vitro diagnostic use.
2. This product has been authorized only for the detection of nucleocapsid protein from SARS-CoV-2, not for any other viruses or pathogens.
3. Proper sample collection and handling are essential for correct results.
4. Do not touch swab tip when handling the swab sample.
5. Leave test card sealed in foil pouch until just before use. Do not use if pouch is damaged or open.
6. Do not use kit past its expiration date.
7. Do not mix test card and sample extraction solution from different kit lots.
8. All kit components are single use items. Do not use with multiple specimens. Do not reuse the used test card.

STORAGE AND STABILITY

Kits should be stored in 2°C–30°C in a cool, dark, dry place preservation, valid for 18 months, forbidden to store under 2°C and avoid using expired products. Manufacture date (MED) and Expiry date (EXP) marked on the label.

TEST PROCEDURE

Do not open the pouch until you are ready to perform a test, and the single-use test is suggested to be used under low environmental humidity (REH>70%) for 1 hour.

Before performing the test, you must read the instruction manual of the product completely, and plaque balance the test cards and sample extraction solution to room temperature (18°C–26°C) before the test. Do not perform the test only when the reagent was equilibrated to room temperature (18°C–26°C) to avoid affecting the accuracy of the experimental results.

1. Open your test kit and you should have:

   1. Test card in a sealed foil pouch, 1 Tube with sample extraction solution and 1 Swab.
   2. Open Pouch and place the card on a clean, dry, flat surface.
      **NOTE:** Do not touch any parts on inside of the card.
   3. Open Swab
      Open swab package and take the swab out.
      **NOTE:** Keep fingers away from swab end.
   4. Sample Collection Process
      Do not eat food or beverages, including gum or tobacco, for 30 minutes before sampling. Press the tip of tongue against the roof of jaw to concentrate saliva. Apply the swab under the tongue for at least 10 seconds rotate 5 times or more and soak it completely.
      **NOTE:** False negative results may occur if the saliva is not properly collected.
   5. Open the lower cover of the tube that has been pre-filled with sample extraction solution
   6. Elution of samples from swab
      Place the swab into the sample tube and then completely immerse the swab head in the sample.
      Vigorously mix the solution by rotating the swab forcefully against the side of the tube at least 10 times (while submerged) and squeeze the tube 5 times by hand to ensure that the sample on the sampling swab is fully eluted into the sample extraction buffer.
   7. Snap the swab head and keep it in the tube
   8. Close the lower cover of the tube and waggle tube for 5-6 times
   9. Open the upper cover of the tube
   10. Dispense 100μL (3 drops) of the specimen into the circular well on the card. Close the upper cover of the tube.

11. Wait 15 minutes

NOTE: Do not disturb card during this time. False results can occur if the card is disturbed/moved or test results are read before 15 minutes.

12. Interpret the test results at 15–20 minutes.
Do not interpret the results after 20 minutes.

DISPOSE IN TRASH
The kit components and patient samples should be handled as infectious waste. The kit components must be disposed of in accordance with local disposal regulations.

INTERPRETATION OF TEST RESULTS
There are three types of results possible:

1. **Positive**
   - Both red/purplish test band (T) and red/purplish control band (C) appear in window.

2. **Negative**
   - Only the red/purplish control band (C) appears in window. The absence of a test band (T) indicates a negative result.

3. **Invalid**
   - In case of a negative test result:
     1. Continue to follow all applicable rules regarding contact with others and protective measures.
     2. Even if the test is negative, an infection may be present.
     3. In case of suspicion, repeat the test after 1 - 2 days, as the coronavirus cannot be accurately detected at all stages of an infection.

   **Handling instructions/Actions after the test result**

1. The following reasons may cause false negative results:
   1) Inappropriate sample collection, using other non-matching solution, sample transfer time is too long (more than half an hour), the volume of solution added when eluted the swab is too much, non-standardized elution operation, low virus titer in the sample, these may all lead to false negative results.
   2) Mutations in viral genes may lead to changes in antigen epitope, leading to false negative results.
   3) Analysis the possibility of false positive results:
      1) Inappropriate sample collection, using other non-matching solutions, non-standardized elution operation, these may all lead to false positive results.
      2) Cross-contamination of samples may lead to false positive results.
      3) False negative result from non-specific acid.

2) The test card would invalid if the package was broken. The packaging status must be carefully checked before use.

**LIMITATIONS**
1. The result of the product should not be taken as a confirmed diagnosis, for clinical reference only. Judgement should be made along with RT-PCR results, clinical symptoms, epidemic condition and further clinical data.
2. Due to the limitation of the detection method, the negative result cannot exclude the possibility of infection. The positive result should not be taken as a confirmed diagnosis. Judgement should be made along with clinical symptoms and further diagnosis methods.
3. Positive test results do not rule out co-infections with other pathogens.
4. False negative results are more likely after 8 days or more of symptoms.
5. Negative results from patients with symptom onset beyond 7 days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.
6. This reagent can only qualitatively detect SARS-CoV-2 antigens in human saliva samples. It cannot determine the certain antigen content in the samples.
7. The accuracy of the test depends on the sample collection process. Improper sample collection will affect the test results.
8. False negative results may occur if swabs are stored in their paper sheath after specimen collection.
9. Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
10. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
11. Cross reactions may exist due to the N-protein in SARS has a high homology with the new coronavirus (SARS-CoV-2). However, the interpretation of the results is not affected during seasons without SARS infection.

**PERFORMANCE CHARACTERISTIC**

1. **Analytical Performance**
   1. Limit of detection
      - This kit was confirmed to detect 1.5 x 10^3 TCID_50/mL of SARS-CoV-2 which was isolated from USA-WA/2020, Gamma-irradiated.
   2. Cross reactivity
      - The following viruses and other Microorganisms have no effect on the test results.

<table>
<thead>
<tr>
<th>Potential Cross-Reagent</th>
<th>Test Concentration</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory Syncytial Virus A</td>
<td>1.0 x 10^8 PFU/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus B</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Measles Virus</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Adenovirus Type 3</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Adenovirus Type 7</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Human cytomegalovirus</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Human coronavirus OC43</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Human coronavirus 229E</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Influenza B</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Influenza A</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Mycoplasma pneumonia</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Epstein Barr Virus</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>HCoV-HKU1</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>1.0 x 10^8 TCID_50/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1.0 x 10^9 CFU/mL</td>
<td>No cross reaction</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>1.0 x 10^9 CFU/mL</td>
<td>No cross reaction</td>
</tr>
</tbody>
</table>

1.3. Interfering Substances
The following interfering substances have no effect on the test results.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Active Ingredient</th>
<th>Concentration</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous</td>
<td>Mucin</td>
<td>2.0 % v/w</td>
<td>No interference</td>
</tr>
<tr>
<td>Nasal Spray</td>
<td>Oxymetazoline</td>
<td>12 % v/v</td>
<td>No interference</td>
</tr>
<tr>
<td>Sore Throat Spray</td>
<td>Phenol</td>
<td>15 % v/v</td>
<td>No interference</td>
</tr>
<tr>
<td>Throat Lozenges</td>
<td>Benzocaine, Menthol</td>
<td>0.15% v/v</td>
<td>No interference</td>
</tr>
<tr>
<td>Anti-viral Drug</td>
<td>Ribavirin</td>
<td>12.9 mg/mL</td>
<td>No interference</td>
</tr>
<tr>
<td>Antibacterial, Systemic</td>
<td>Tobramycin</td>
<td>4.0 g/mL</td>
<td>No interference</td>
</tr>
</tbody>
</table>

1.4. High Dose Hock Effect
No high dose hock effect was observed when tested with up to a concentration of 1.6 x 10^6 TCID_50/mL of heat inactivated SARS-CoV-2 virus.

2. Clinical study
Performance of SARS-CoV-2 Ag Test Kit, with the test performed and results interpreted by the home user is similar to performance obtained by test operators with no laboratory experience. The clinical evaluation was performed to compare the results obtained with the SARS-CoV-2 Ag Test Kit and a comparative reverse transcriptase polymerase chain reaction test (Novo Coronavirus 2019-nCoV Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) manufactured by Samsun Biotech Inc.). Among patients, there are 157 positive and 182 negative saliva samples by IVE-PCR confirmed. The presentation of the results of the SARS-CoV-2 Ag Test Kit is as follows.
<table>
<thead>
<tr>
<th>Value</th>
<th>Samples</th>
<th>nCoV/RT-PCR Results</th>
<th>to RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30</td>
<td>46</td>
<td>positive</td>
<td>44/46~95.65% (95%CI:85.47%~98.80%)</td>
</tr>
<tr>
<td>≤36</td>
<td>157</td>
<td>positive</td>
<td>142/157~90.45% (95%CI:84.84%~94.12%)</td>
</tr>
<tr>
<td>&gt;40</td>
<td>182</td>
<td>negative</td>
<td>181/182~99.45% (95%CI:96.95%~99.90%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days</th>
<th>Number of samples</th>
<th>2019 nCoV RT-PCR Results</th>
<th>SARS-CoV-2 antigen test result as compared to RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤7</td>
<td>89</td>
<td>positive</td>
<td>85/89~95.51% (95%CI:89.01%~98.24%)</td>
</tr>
<tr>
<td>≤14</td>
<td>116</td>
<td>positive</td>
<td>107/116~92.24% (95%CI:85.91%~95.86%)</td>
</tr>
<tr>
<td>&gt;14</td>
<td>41</td>
<td>positive</td>
<td>35/41~85.37% (95%CI:71.56%~93.12%)</td>
</tr>
</tbody>
</table>

Sensitivity: 90.45% (95%CI:84.84%~94.12%) for CT values ≤36
Sensitivity: 95.51% (95%CI:89.01%~98.24%) for onset of symptoms within 7 days
Specificity: 99.45% (95%CI:96.95%~99.90%)

3. Human Factors Study
Watnim conducted a human factor’s study to evaluate whether home user patients or caregivers (lay user) could perform the test and accurately interpret test results from the SARS-CoV-2 Ag Card.
In this study, a total of 50 lay users, age 15 and older with either good or corrected vision (far/near-sighted or wear bifocals) participated in a 30-minute session including an introduction, a product overview, and simulated use cases of SARS-CoV-2 Ag Test Kit result interpretation. Participants were asked to read and interpret a panel of 7 different SARS-CoV-2 Ag Card test results, including high positive, low positive, negative and invalid.
46/50 participants described the process of reading and interpreting the test card results as being easy. However, 4/50 of the participants commented that it was difficult to see some of the fainter line conditions.
A total of 350 trials were recorded in this study. Participants were able to perceive and interpret the results correctly for 327 trials, or 92.94% of the time. Positive results with stronger intensity lines were easier to read than the positive lines with less intensity.
After the human factors evaluation, participants were asked for their overall impressions of the instructional materials they were provided. Nearly all participants (49/50) thought the instructions were straightforward and easy to understand and follow.

4. Usability Study
Watnim conducted a study to evaluate whether a home user can read the instructions and successfully perform the test steps for the SARS-CoV-2 Ag Card test, including swab collection at home, and correctly interpreting the results.
120 home users, including individuals (n=60) and caregivers (n=60), participated in the study. Each individual or caregiver pair participated in a 30-minute session with an instruction. The usability evaluation session included one simulated use of the SARS-CoV-2 Ag Test Kit.
97.5% (117 out of 120) home users produced a valid result (all negatives) and 3 participants produced an invalid result. (The causes of the invalid tests were insufficient amount of reagent added, and damage to the test strip). 117 out of 120 participants interpreted their test result correctly and 3 participants interpreted their result incorrectly (where they perceived a faint line in the sample window (as positive) when there was none (all results were verified by the study moderator).
The individual home user group completed 99.4% (133/134) of the total tasks/steps correctly. The caregiver home user group completed 99.3% (133/134) of the total tasks/steps correctly.

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